Morphological, Pathogenic, and Genetic Comparisons of *Colletotrichum graminicola* Isolates from Poaceae

Marsha Browning, Lisa V. Rowley, Peiyu Zeng, Joel M. Chandlee, and Noel Jackson, Department of Plant Sciences, University of Rhode Island, Kingston 02881

ABSTRACT

Browning, M., Rowley, L. V., Zeng, P., Chandlee, J. M., and Jackson, N. 1999. Morphological, pathogenic, and genetic comparisons of *Colletotrichum graminicola* isolates from Poaceae. Plant Dis. 83:286-292.

Isolates of *Colletotrichum graminicola* from annual blue grass and creeping bent grass were investigated for their morphological characteristics, host specificity, and genetic relatedness. One isolate from maize and one from sorghum (*C. sublineolum*) were included for comparison. Recently isolated cultures of *C. graminicola* from annual blue grass were readily distinguished from those isolated from creeping bent grass on the basis of pigmentation. Differences in appressoria size and shape were found only between the turf grass isolates and those from maize and sorghum. Spore length varied significantly between host groups. Differences in host range and virulence were also apparent. In general, isolates from creeping bent grass incited disease on both creeping bent grass and annual blue grass, while those from annual blue grass essentially were limited to host. Random amplified polymorphic DNA (RAPD) marker analysis of *C. graminicola* isolates from turf grass revealed that a high degree of genetic similarity exists among isolates recovered from the same host, but exceptions were found. Therefore, an absolute distinction between isolates recovered from two turf grass hosts could not be made based on RAPD markers.

Additional keyword: biotype

Anthrachnose disease associated with *Colletotrichum graminicola* has been reported on a broad range of grasses and cereals worldwide. The ubiquitous occurrence of this fungus on senescing and dead members of Poaceae includes amenity grasses, but the number of turf species subject to severe damage is limited. The historical and sometimes controversial record of *C. graminicola* as a turf grass pathogen has been reviewed (17). Anthracnose is now accepted as a particularly serious disease of annual blue grass (*Poa annua*) and, increasingly, a problem disease of bent grass (*Agrostis* spp.) turfs on golf courses in the northern United States, Canada, and Europe.

Disease symptoms may include lesioning or blighting of aerial parts (foliage anthracnose) and a basal stem, crown, and root rot (basal stem rot anthracnose, BSR). Infection of the stem bases and crowns is

invariably fatal and severe thinning of the turf occurs (17). Annual blue grass turf demonstrates the most distinctive BSR symptoms. Typically, small clusters of infected plants turn yellow to give the turf a measled appearance. Spots soon coalesce and older leaves turn brown, but the central shoots of infected tillers often remain bright yellow to orange-red in color and detach easily to reveal a blackened base. In managed swards of other turf grasses, unthriftiness and premature senescence of older leaves mark the advance of foliage infection. Progression to the BSR stage is of increasing incidence in bent grass turf. Diffuse to well-defined pale yellow to straw-colored patches, varying in size and shape from a few square centimeters to several square meters, develop as the pathogen gradually colonizes creeping stems and basal parts of bent grass plants. Blackening of the infected tissues is often noticeable, especially on the stolons of creeping bent grass.

In mixed turf swards, some species may be subject to anthracnose while others remain healthy. Smiley et al. (16) noted that the fine leaf fescue (*Festuca* spp.) component of a turf mix became infected while the accompanying Kentucky blue grass (*Poa pratensis*) remained disease free. It is commonly observed in annual blue grass-creeping bent grass greens that one or the other of the grasses is infected, but seldom both. In 1996, 55% of the anthracnose-infected turf samples submitted

to our diagnostic laboratory were composed of annual blue grass, 41% of creeping bent grass, and only 3% represented a dual infection. These observations suggested some degree of host specificity among *C. graminicola* isolates, and the possibility of biotypes of the pathogen whereby differences in host range and virulence would be due to differences in genetic makeup.

Sutton (18,19) proposed that isolates of *C. graminicola* from sorghum (*Sorghum bicolor*) and maize (*Zea mays*) be placed in separate species based on morphological differences. The sorghum and maize isolates were designated *C. sublineolum* and *C. graminicola*, respectively, largely based on appressorial characteristics. Where differences in morphology do not exist, host range as well as the severity of infection can be used as a tool to identify biotypes or races of a fungal species (21). More recently, Vaillancourt and Hanau (20) confirmed Sutton's findings using a random amplified polymorphic DNA (RAPD) fingerprinting analysis. Sheriff et al. (14) provided further confirmation through ribosomal DNA (rDNA) sequence analysis. Previous work has also shown the utility of using RAPD markers for differentiating *C. graminicola* isolates of sorghum (6).

The objective of this investigation was to confirm the premise that host specificity occurs among *Colletotrichum* isolates from turf, particularly among bent grasses and annual blue grasses, and to determine if this variation in host range can be attributed to differences in genetic makeup of the fungus. The criteria for such separation were differences in morphology, host specificity, and genetic organization.

MATERIALS AND METHODS

C. graminicola cultures used in these experiments were isolated from turf grass samples received in our diagnostic lab; 11 cultures were isolated from annual blue grass and 17 from creeping bent grass. Origin and host plant for each isolate are listed in Table 1. Cultures were maintained on half-strength potato dextrose agar (PDA; Difco Laboratories, Detroit) at room temperature (23 to 25°C) under continuous fluorescent light to promote sporulation (9). One stock culture of *C. graminicola*, isolated from maize, and one of *C. sublineolum*, isolated from sorghum (supplied by R. L. Nicholson, Purdue Uni-

Corresponding author: N. Jackson
E-mail: noeljack@uri.edu

Contribution 3671 from the Rhode Island Agricultural Experiment Station, Kingston.

This research was supported by the Tristate Turf Research Foundation.

Accepted for publication 15 November 1998.

Publication no. D-1999-0125-01R
© 1999 The American Phytopathological Society

versity) were used for comparison with the cultures isolated from turf grass.

Morphological characteristics. Colony appearance (form and pigmentation), the size and shape of appressoria, and spore size were investigated as possible indicators of relatedness among all of the isolates. Colony pigmentation on half-strength PDA was noted 2 weeks after isolation for each culture.

Appressoria. Blocks (1.5 by 1.5 by 0.2 cm) of half-strength PDA were placed on sterile slides, inoculated on all 4 sides with mycelia from one isolate of *C. graminicola*, and covered with a sterile cover slip. Two slide cultures were prepared for each isolate. Cultures were incubated in a moist chamber for 1 to 3 weeks at 23°C under continuous fluorescent light until appressoria had developed. The agar block was then removed, leaving a film of appressoria and spores attached to the glass. Both slides and coverslips were treated with lactophenol and mounted to provide 4 slides/isolate. A total of 10 apical appressoria, selected from each of 3 slides/isolate, were measured at 600× according to protocol used by Sutton (18); length was measured from "truncate base to the farthest apex" and breadth measurement "taken at right angles to the length axis across widest part of the appressorium, including lobes." Shape and color were also noted. Dimensions of appressoria and spores were compared statistically among individual isolates and host group with analysis of variance ($P = 0.05$). Mean separation was carried out with the least significant difference test (LSD).

Spores. Spore size was also determined by measuring ten spores from each of three slides/isolate. Differences were determined as previously described.

Host specificity. A total of 22 isolates of *C. graminicola* were chosen, including 8 from annual blue grass, 12 from creeping bent grass, 1 from maize, and 1 isolate of *C. sublineolum* from sorghum, and evaluated for pathogenicity on 6-week-old annual blue grass seeded at a rate of 40 g/m² and cv. Penncross creeping bent grass seeded at 26 g/m². The grasses, grown in 7.6-cm square pots in Terragreen (Oil Dry Corporation, Alpharetta, GA), were inoculated with 1.5 ml of a spore suspension per pot using a DeVilbiss atomizer (DeVilbiss, Inc., Pittsburgh). Spore concentrations ranged from 5.1×10^4 to 6.0×10^5 spores/ml. Treatments were replicated six times. Following foliar application of the spores, additional distilled water was syringed on the leaves until runoff to wash spores down into the crown region. The treated grasses were transferred to a greenhouse bench and maintained on moistened Troy Flo Thru capillary matting (Troy Mills, Inc. Troy, NH) in a shaded plastic tent, designed to ensure high humidity and temperature (2,4) and low light (9). Temperature and relative humidity were moni-

Fig. 1 Dendrogram based on random amplified polymorphic DNA patterns showing the relationships among 24 isolates of *Colletotrichum* spp. using a total of 12 arbitrary primers. The dendrogram was constructed by the NTSYS program (12) using the unweighted pair group method with arithmetic based on simple matching coefficients. Isolates 1 to 12 and 22 were recovered from creeping bent grass (CBG) and isolates 13 to 21 were recovered from annual blue grass (ABG).

Table 1. *Colletotrichum* spp. isolates used in this study, their host species, and sources

Isolate no.	Host species	Origin
1	<i>Agrostis palustris</i>	Country Club of Farmington, Farmington, CT
2	<i>Agrostis palustris</i>	Back O' Beyond, Brewster, NY
3	<i>Agrostis palustris</i>	Passaconway Country Club, Litchfield, NH
4	<i>Agrostis palustris</i>	Piping Rock Club, Locust Valley, NY
5	<i>Agrostis palustris</i>	River Run Country Club, Berlin, MD
6	<i>Agrostis palustris</i>	Pemaquid Point Lighthouse, Pemaquid, ME
7	<i>Agrostis palustris</i>	Cohasse Country Club, Southbridge, MA
8	<i>Agrostis palustris</i>	Silver Spring Country Club, Ridgefield, CT
9	<i>Agrostis palustris</i>	Pine Acres Golf Course, Bellingham, MA
10	<i>Agrostis palustris</i>	Wethersfield Country Club, Wethersfield, CT
11	<i>Agrostis palustris</i>	Atlantic Country Club, Plymouth, MA
12	<i>Agrostis palustris</i>	Stonington Country Club, Stonington, CT
22	<i>Agrostis palustris</i>	Metacomet Country Club, E. Providence, RI
25	<i>Agrostis palustris</i>	Ipswich Country Club, Ipswich, MA
26	<i>Agrostis palustris</i>	Aspetuck Valley Country Club, Weston, CT
27	<i>Agrostis palustris</i>	Greenwich Country Club, Greenwich, CT
28	<i>Agrostis palustris</i>	Kingsway Golf Club, Yarmouthport, MA
13	<i>Poa annua</i>	Alpine Country Club, Cranston, RI
14	<i>Poa annua</i>	Kebo Valley Country Club, Bar Harbor, ME
15	<i>Poa annua</i>	Country Club of Madison, Madison, CT
16	<i>Poa annua</i>	Sands Point Club, Sands Point, NY
17	<i>Poa annua</i>	Yale Golf Course, New Haven, CT
18	<i>Poa annua</i>	Country Club of Darien, Darien, CT
19	<i>Poa annua</i>	home lawn, Wakefield, RI
20	<i>Poa annua</i>	Worcester Country Club, Worcester, MA
21	<i>Poa annua</i>	Rockland Park Golf Course, St. John, N.B., Canada
29	<i>Poa annua</i>	Quaker Ridge, Scarsdale, NY
30	<i>Poa annua</i>	Washington County Sod, W. Kingston, RI
23	<i>Zea mays</i>	'CgMz' Texas, R. L. Nicholson, Purdue Univ.
24	<i>Sorghum bicolor</i>	'Cg Tx430 BB' Texas Brazo, R. L. Nicholson, Purdue

tored with a hygrothermograph. Following 3 weeks of incubation in the tent and 1 week on a greenhouse bench, 25 grass plants were selected from each pot and evaluated microscopically for signs of foliar infection, determined by the presence of acervuli with setae, and symptoms of basal stem rot, recognizable by blackening at the base of central shoots. Data, expressed as percent infected plants, was subjected to analysis of variance ($P = 0.05$); means were separated out with LSD. Greenhouse pathogenicity trials were conducted twice.

Generation of teleomorphic state. Politis (11) described the teleomorph derived in vitro from maize isolates of *C. graminicola* and designated it as *Glomerella graminicola* Politis. Vaillancourt and Hanau (20) successfully produced the perfect state from sorghum isolates of the fungus, again under laboratory culture. The teleomorph has not been reported to occur in nature and never from turf grass isolates of *C. graminicola*. Following the procedure outlined by Vaillancourt and Hanau (20), matings between all possible combinations of annual blue grass and creeping bent grass isolates were attempted on sterilized maize leaves to determine possible compatibility.

Briefly, 8-cm lengths of maize leaves were autoclaved (15 min, 121°C), then arranged in a cross pattern in petri dishes

containing sterile moistened filter paper or Sach's agar (a low-pH mineral medium; 8,15). Plugs from 23 monoconidial isolates of *C. graminicola* (12 creeping bent grass, 10 annual blue grass, 1 maize) and 1 sorghum isolate (*C. sublineolum*) were arranged four to a dish in all possible combinations and replicated three times for a total of 648 plates. The dishes were maintained at approximately 20°C under continuous fluorescent light for 3 weeks, then examined microscopically for the presence of the sexual state (ascocarps and ascospores).

RAPD analysis. A total of 24 isolates of *C. graminicola* from four different hosts (Table 1 and Fig. 1) were used in this study. Fungal genomic DNA was extracted from dried mycelia of all 24 isolates of *C. graminicola* using standard methods (13): fungal cells were disrupted using a sodium dodecyl sulfate (SDS)-containing extraction buffer (100 mM Tris-Cl, 50 mM EDTA, 500 mM NaCl, and 1% SDS, pH 8.0); genomic DNA was precipitated from the supernatant by the addition of isopropanol and then further purified in a CsCl density gradient (13).

RAPD assay conditions were essentially as described by Williams et al. (22), using 0.2 mM each of dATP, dTTP, dGTP, and dCTP, 0.1% gelatin, 10 ng genomic DNA, 60 ng each of 12 10-mer primers (GCGGCCCTCT, UBC 356; TGCGCC-

GCTA, UBC 384; CAGGCCCTTC, TURF 1; TGCCGAGCTG, TURF 2; CACAACGGGT, UBC 352; CTCTCCAGGC, UBC 360; GGGCTGTGCC, UBC 397; CTCACTTGGG, UBC 339; GCGAACCTCG, UBC 391; CGCTGTGCC, UBC 387; AGGCCAAATG, UBC 357; and GCGTGACCCG, UBC 345), 0.72 units of *Taq* polymerase, 1.5 mM MgCl₂, and reaction buffer to a total volume of 12 ml. The reaction mixture was incubated in a Perkin-Elmer GeneAmp polymerase chain reaction (PCR) system programmed for 44 cycles of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C, followed by one cycle of 1 min at 94°C, 1 min at 36°C, and 5 min at 72°C. All reactant products, along with negative controls (the reaction mixture without genomic DNA added), were analyzed by gel electrophoresis in 1.4% agarose in Tris-Cl EDTA buffer stained with ethidium bromide (13).

RAPD banding patterns corresponding to the 24 *C. graminicola* isolates were scored visually for the presence or absence of particular DNA bands. A similarity matrix and cluster analysis was performed using the numerical taxonomy software package NTSYS (12).

RESULTS

Morphological characteristics. *Colony appearance.* Recently isolated cultures of *C. graminicola* from annual blue grass

Table 2. Dimensions of appressoria from *Colletotrichum* spp. isolates^a

Isolate, source ^b	Appressoria length (μm)		Appressoria width (μm)		Description
	Range	Mean ± SD	Range	Mean ± SD	
CBG					
Aspetuck	10–23	14.4 ± 3.0	6–13	9.3 ± 1.6	Smooth
Atlantic	9–15	11.6 ± 2.1	6–13	9.0 ± 2.3	Smooth
Back O'Beyond	10–19	14.3 ± 2.5	6–13	8.9 ± 1.8	Smooth
Farmington	9–26	17.1 ± 4.3	8–15	10.7 ± 2.5	Irregular
Greenwich	9–19	14.1 ± 2.7	6–15	9.2 ± 2.1	Irregular
Ipswich	10–19	14.5 ± 2.1	6–15	10.1 ± 2.0	Smooth
Kingsway	10–19	14.0 ± 2.6	8–15	10.0 ± 2.1	Smooth
Passaconway	9–18	13.0 ± 2.3	8–14	9.5 ± 1.9	Variable
Pemaquid	10–25	15.3 ± 3.0	5–13	9.6 ± 1.9	Smooth; some lobate
Pine Acres	10–23	16.3 ± 3.0	8–13	9.6 ± 1.6	Smooth; some irregular
River Run	9–21	15.4 ± 3.5	8–14	9.6 ± 1.5	Smooth; some irregular
Silver Spring	8–23	14.1 ± 3.3	8–15	9.7 ± 1.8	Smooth; some irregular
Stonington	10–18	13.8 ± 1.6	9–13	10.4 ± 1.7	Smooth
Wethersfield	13–25	17.3 ± 2.9	8–15	10.8 ± 2.0	Smooth
Mean	...	14.6 ± 3.2	...	9.7 ± 2.0	...
ABG					
Alpine	8–19	11.6 ± 2.5	8–14	10.1 ± 1.5	Smooth; some lobate
Madison	9–15	11.6 ± 1.7	5–11	9.1 ± 1.5	Smooth; some lobate
New Brunswick	8–19	13.5 ± 3.0	8–16	11.8 ± 2.4	Smooth
Quaker Ridge	11–24	15.8 ± 3.2	5–11	7.9 ± 1.5	Smooth to irregular; not lobate
Sands Point	8–14	10.9 ± 1.6	6–13	8.8 ± 1.6	Smooth; mostly intercalary
Wakefield	10–19	14.6 ± 2.4	6–13	9.4 ± 1.7	Smooth
Wash. Co. Sod	8–19	14.7 ± 2.6	6–21	10.5 ± 3.1	Smooth
Worcester	8–15	11.6 ± 1.9	6–13	9.1 ± 1.7	Smooth; some lobate
Yale	10–20	16.1 ± 2.8	6–16	9.4 ± 2.1	Lobate, irregular
Mean	...	13.4 ± 3.1	...	9.6 ± 2.2	...
Maize	19–38	26.5 ± 4.4	13–32	23.2 ± 5.0	Multi-lobate; irregular
Sorghum	10–25	15.9 ± 3.7	6–25	13.7 ± 4.6	Smooth
LSD	...	1.4	...	1.2	...

^a $F = 34.16$ and 47.73 , $df = 24$ and 714 , $P < 0.01$.

^b CBG = creeping bent grass and ABG = annual blue grass; LSD = least significant difference.

were readily distinguished from those isolated from bent grass on the basis of pigmentation. On half-strength PDA, annual blue grass isolates consistently produced dense, floccose colonies that varied in mycelial pigmentation from pink with beige to pink with olive green. Colonies from creeping bent grass were never pink in color; instead, the appressed mycelia were black-gray in appearance. After numerous transfers or long-term storage, the distinctive features were lost. Growth of all the colonies became appressed and aged to a light gray color. The maize and sorghum isolates were similar in appearance; fluffy white mycelium soon developed numerous orange, spore-bearing conidiomata produced in concentric rings. Fructifications of the sorghum isolate tended to be darker orange in color than those of maize under continuous fluorescent light. The maize and sorghum isolates produced spores within 1 week of transfer, whereas the turf grass isolates required nearly 2 weeks to sporulate.

Appressoria. The appressoria of *C. graminicola* isolated from turf grass varied in size and shape from round to lobate with some irregular (Table 2). The maize isolate produced appressoria which were distinct from those from turf grass, being very large and irregularly shaped. Appressoria from the sorghum isolate were rounded and smaller in size, close in dimension to those from turf grass isolates. Dimensions of appressoria, both length and width, were found to be statistically different ($P < 0.01$; $F = 130.5$ and 110.6 , respectively) among sorghum, maize, and the turf grass isolates grouped by host. Comparing dimensions of appressoria from annual blue grass isolates with those from creeping bent grass, only appressorial length was statistically different.

Spores. Spores of all isolates were allantoid, lunate, to falcate, and were variable in size for each isolate (Table 3). Comparing isolates from annual blue grass, creeping bent grass, maize and sorghum, spore length, but not width, was found to be statistically different between groups ($P < 0.01$; $F = 73.1$).

Host specificity. In greenhouse pathogenicity trials, anthracnose leaf infection, basal stem rot, or both, were established for all of the isolates tested on their host of origin. Symptoms developed 2 to 3 weeks following inoculation when grasses were maintained under high humidity (70 to 90%) and high temperature. Mean temperature for the incubation period was 30°C (range 15 to 41°C) in the first trial and 26.5°C (range 10 to 44°C) in the second trial. Infection overall was lower in the second trial, presumably due to the increasing age of the cultures and variable temperature. Variation in host specificity and disease severity was demonstrated (Tables 4 and 5). In general, creeping bent grass isolates infected both creeping bent

grass and annual blue grass. Annual blue grass isolates were efficient in colonizing annual blue grass but were much less able to infect creeping bent grass. Annual blue grass isolates caused more severe basal stem rot on annual blue grass than did the creeping bent grass isolates on their respective hosts. Creeping bent grass isolates on annual blue grass caused only minor basal stem rot symptoms. Inoculation with maize or sorghum isolates resulted in foliar infection of annual blue grass, but not creeping bent grass.

Generation of teleomorphic state. Despite an extended period of incubation (terminated at 8 weeks), no ascocarp production was observed in plates of any of the combinations of isolates. Structures resembling ascocarp initials were frequently observed but they did not progress to maturity.

RAPD analysis. From the 12 primers used in this study, a total of 156 scorable bands were generated and included in the similarity and cluster analysis (Fig. 1). These bands allowed the similarity among *Colletotrichum* isolates from annual blue grass, creeping bent grass, corn, and sorghum to be tested.

There is approximately 60% similarity between *Colletotrichum* isolates from sorghum and corn (Fig. 1), similar to a value determined in a separate study (6), and turf grass isolates share approximately 57%

similarity with isolates from corn and sorghum. This suggests that *Colletotrichum* isolates from corn and sorghum are slightly more related to each other than to those from turf grass. There are six distinct clusters within the isolates from turf grass (Fig. 1). Of 12 *C. graminicola* isolates from bent grass, 7 fall into group III and share over 90% similarity, 5 of 9 *C. graminicola* isolates from annual blue grass fall into group V and share over 90% similarity, and 4 of 12 *C. graminicola* isolates from bent grass fall into group IV and share over 80% similarity. Of 9 *C. graminicola* isolates, 2 from annual blue grass and 1 from bent grass fall into group VI and share over 90% similarity. Two additional small groups are found in this cluster tree: only isolate 1 from bent grass shares over 80% similarity with group II (isolates 17 and 18) from annual blue grass. Both group I and II share over 72% similarity with group III from bent grass.

There were 2 unique RAPD bands identified for *Colletotrichum* isolates from turf grass, 11 from corn, and 11 from sorghum (Table 6). There were two common bands between *Colletotrichum* isolates from corn and sorghum, one between isolates from turf grass and corn, and none between those from turf grass and sorghum.

No host-specific bands of *C. graminicola* were associated with all the isolates from either creeping bent grass or annual

Table 3. Dimensions of spores from *Colletotrichum* spp. isolates^a

Isolate, source ^b	Spore length (µm)		Spore width (µm)	
	Range	Mean ± SD	Range	Mean ± SD
CBG				
Aspetuck	15–29	19.8 ± 3.3	3.8–6.3	5.00 ± 0.5
Atlantic	20–28	22.7 ± 1.9	...	5.04 ± 0.0
Back O' Beyond	11–20	16.2 ± 2.6	1.3–6.3	4.90 ± 1.3
Farmington	24–33	28.6 ± 2.3	...	3.78 ± 0.0
Greenwich	15–30	22.1 ± 4.1	...	5.04 ± 0.0
Kingsway	19–30	24.2 ± 2.4	...	5.04 ± 0.0
Ipswich	6–33	24.5 ± 4.4	3.8–6.3	5.04 ± 0.3
Passaconway	20–29	23.3 ± 2.4	3.8–5.0	5.00 ± 0.2
Pemaquid	20–28	23.9 ± 2.0	...	5.04 ± 0.0
Pine Acres	14–32	21.4 ± 3.8	3.8–5.0	4.07 ± 0.5
Silver Spring	10–25	19.9 ± 4.0	5.0–6.3	5.25 ± 0.5
Stonington	15–27	21.7 ± 3.0	3.8–6.3	5.08 ± 0.4
Wethersfield	21–32	25.5 ± 2.3	...	5.04 ± 0.0
Mean	...	22.6 ± 4.2	...	4.9 ± 0.6
ABG				
Alpine	15–27	20.8 ± 2.4	...	5.04 ± 0.0
Madison	23–32	25.4 ± 2.2	...	5.04 ± 0.0
New Brunswick	21–32	26.1 ± 2.7	5.0–6.3	5.10 ± 0.2
Quaker Ridge	15–30	23.9 ± 3.8	3.8–5.0	4.70 ± 0.6
Sands Point	11–33	22.8 ± 4.2	3.8–6.3	5.00 ± 0.6
Wash. Co. Sod	14–35	24.7 ± 5.5	3.8–6.3	5.80 ± 0.7
Wakefield	21–32	26.1 ± 2.7	3.8–6.3	5.04 ± 0.5
Worcester	15–28	23.1 ± 2.5	3.8–6.3	5.04 ± 0.5
Yale	15–29	24.1 ± 3.3	3.8–5.0	4.07 ± 0.5
Mean	...	24.1 ± 3.7	...	4.98 ± 0.6
Maize	13–44	34.8 ± 7.1	...	5.04 ± 0.0
Sorghum	24–38	30.4 ± 3.1	...	5.04 ± 0.0
LSD	...	1.8	...	0.21

^a $F = 34.92$ and 30.09 , $df = 23$ and 685 , $P < 0.01$.

^b CBG = creeping bent grass and ABG = annual blue grass; LSD = least significant difference.

^c All measurements were the same.

blue grass. However, a summary of the DNA bands from a majority of *C. graminicola* isolates from either creeping bent grass or annual blue grass indicate significant differences in the isolates (Table 7).

Band A, generated by the primer UBC 345, was present in 11 of 12 *C. graminicola* isolates from creeping bent grass but none of the isolates from annual blue grass. There were collectively four bands pro-

duced by primers UBC 352 (G), UBC 391 (J), and UBC 345 (B and D) present in 7 of 9 *C. graminicola* isolates from annual blue grass and, with one exception (UBC 391, band J), no isolates from creeping bent grass. The primer UBC 360 produced a band E present in 11 of 12 *C. graminicola* isolates from bent grass and 2 of 9 (isolates 17 and 18) from annual blue grass. Two annual blue grass isolates (isolates 17 and 18) appeared to be more closely related to *C. graminicola* isolates from creeping bent grass than other isolates from annual blue grass (Fig. 1). Again, these may be potential diagnostic bands for isolates from creeping bent grass.

Isolate 11 from creeping bent grass shares a common band (J, produced by primer UBC 391) with 7 of 9 *C. graminicola* isolates from annual blue grass but not with other isolates from bent grass (Table 7). Isolates 17 and 18 from annual blue grass share a common band (E, produced by primer UBC 360) with 11 of 12 isolates from creeping bent grass but with none from annual blue grass. These observations suggest that these three isolates (numbers

Table 4. Pathogenicity trial 1. Percent infection by *Colletotrichum* spp. isolates on annual blue grass (ABG) and creeping bent grass (CBG) following foliar application of spores and incubation in a humidity chamber^a

Isolate, source	Annual blue grass		Creeping bent grass	
	Foliar	BSR	Foliar	BSR
CBG				
Atlantic	41	15	4	0
Back O'Beyond	56	3	31	0
Cohasse	4	0	2	0
Farmington	4	0	0	0
Ipswich	3	0	61	19
Metacomet	14	1	2	0
Passaconway	36	6	77	75
Pemaquid	51	1	14	0
Pine Acres	38	3	21	0
Silver Spring	75	0	35	0
Stonington	99	36	45	2
Wethersfield	55	19	41	0
ABG				
Alpine	95	77	1	0
Kebo	5	1	0	0
Madison	61	18	0	0
New Brunswick	99	85	0	0
Sands Point	91	49	0	0
Wakefield	73	26	0	0
Worcester	99	85	0	0
Yale	67	17	0	0
Maize	58	1	0	0
Sorghum	40	8	0	0
Control	0	0	0	0
LSD	18	16	15	6

^a $F = 27.97, 24.26, 18.41, \text{ and } 55.99$; $df = 21 \text{ and } 105$; $P < 0.01$; LSD = least significant difference; BSR = basal stem rot anthracnose.

Table 5. Pathogenicity trial 2. Percent infection by *Colletotrichum* spp. isolates on annual blue grass (ABG) and creeping bent grass (CBG) following foliar application of spores and incubation in a humidity chamber^a

Isolate, source	Annual blue grass		Creeping bent grass	
	Foliar	BSR	Foliar	BSR
CBG				
Atlantic	0	0	1	0
Back O'Beyond	30	0	48	0
Cohasse	1	1	1	0
Farmington	0	0	1	0
Ipswich	27	1	88	41
Passaconway	0	0	2	1
Pemaquid	16	3	23	0
Pine Acres	11	11	3	0
Silver Spring	27	1	63	1
Stonington	30	10	4	1
Wethersfield	12	2	84	44
ABG				
Alpine	15	5	0	0
Kebo	3	3	1	0
Madison	37	70	0	0
New Brunswick	56	11	1	0
Sands Point	17	7	4	0
Wakefield	77	51	13	0
Worcester	8	3	0	0
Yale	65	69	6	0
Maize	0	0	1	0
Sorghum	0	0	0	0
Control	0	0	0	0
LSD	18	13	12	9

^a $F = 12.18, 23.77, 42.01, \text{ and } 17.27$; $df = 20 \text{ and } 100$; $P < 0.01$; LSD = least significant difference; BSR = basal stem rot anthracnose.

Table 6. Unique bands for *Colletotrichum* spp. isolates from turf grass, corn, and sorghum^a

Primer	Band ^b	Isolates, source		
		Grass	Corn	Sorghum
Turf 1	N	–	+	–
Turf 2	A	–	+	–
UBC 356	A	–	+	+
UBC 356	D	–	–	+
UBC 356	E	+	+	–
UBC 356	H	–	–	+
UBC 356	K	–	+	–
UBC 384	E	–	–	+
UBC 384	G	–	+	–
UBC 384	I	–	+	–
UBC 384	J	+	–	–
UBC 352	E	–	–	+
UBC 397	C	–	+	–
UBC 397	E	–	–	+
UBC 397	J	–	+	–
UBC 339	A	–	+	–
UBC 339	C	–	–	+
UBC 391	A	–	+	+
UBC 391	K	–	–	+
UBC 391	M	–	+	–
UBC 387	G	–	+	–
UBC 387	Q	+	–	–
UBC 387	R	–	–	+
UBC 357	B	–	+	–
UBC 357	E	–	–	+
UBC 357	H	–	–	+
UBC 345	E	–	–	+

^a There were 2 unique bands for *Colletotrichum* spp. isolates from turf grass, 11 from corn, and 11 from sorghum. The *C. graminicola* isolates from turf grass share one common band with that from corn and none with that from sorghum, while two common bands are shared by *Colletotrichum* spp. isolates from corn and that from sorghum (+ = presence and – = absence).

^b Bands were assigned letter designations sequentially (beginning with A) from those closest to the origin of migration to those furthest away.

11, 17, and 18) may have at least some genetic relationship with isolates from the alternative host and may possibly cause serious anthracnose disease in cross-infection experiments. This was demonstrated with isolate 11 (Atlantic) which was more virulent on its alternative host, but isolate 17 (Yale) was only weakly pathogenic on its alternative host.

DISCUSSION

A comparison of morphological features of *C. graminicola* and *C. sublineolum* does not provide a reliable means for differentiation of the species. Colonies from maize, sorghum, annual blue grass, and creeping bent grass, when plated on half-strength PDA, initially look distinct from each other. However, culture changes appear with age and repeated subculturing.

Although some differences in appressoria and spore mean dimensions were found to be statistically significant, practical and confident separation of the *Colletotrichum* isolates from sorghum, annual blue grass, and creeping bent grass would be difficult due to the overlap observed in the range of measurements. Appressoria shape and size in the maize isolate made it readily distinguishable from the sorghum isolate (18,20) and also from the turf grass isolates.

Host specificity was evident in the greenhouse pathogenicity trials. While some variability in virulence among isolates and susceptibility among hosts is to be expected (2), the limited host range exhibited by the annual blue grass isolates suggests the existence of biotypes within *C. graminicola* affecting turf grass. The terms biotype and physiological race are similar and have been used interchangeably;

biotype refers to similarities in genetic makeup, while the connotation of race denotes morphologically similar isolates within a species which differ in some way (i.e., physiological, biochemical, or pathogenic differences; 7). Physiological races based on host specificity have been reported from *C. graminicola* isolated from maize (5,9) and *C. sublineolum* isolated from sorghum (1,10).

We attempted to determine if turf grass isolates of *C. graminicola* were reproductively compatible within the group and with a corn and sorghum isolate. The teleomorph of a *C. graminicola* isolate from maize was described by Politis (11) as *Glomerella graminicola*. It has not yet been observed in nature. Vaillancourt and Hanau (20) were successful in the production of this teleomorph using several isolates from sorghum. They reported that *C. graminicola* (and *C. sublineolum*) isolates are mostly heterothallic, requiring an exchange of genetic material with a compatible strain. Crosses between maize isolates and sorghum isolates failed to produce ascocarps, confirming further that these isolates are reproductively separate. Correll and Guerber (3) demonstrated the existence of two vegetatively compatible groups in *C. dematium* f. *spinaciae*, the spinach anthracnose pathogen, through the use of nitrate non-utilizing mutants. We were unable to achieve the production of the sexual state with any combination of our turf grass isolates and thus are unable to draw any conclusions concerning reproductive compatibility at this time.

The *C. graminicola* isolates from turf grass used in this study were collected from various geographic locations in the

northeastern United States. The RAPD fingerprinting method revealed clear, but not definitive, distinctions among isolates of *C. graminicola* recovered from different hosts. Isolates 17 and 18, both of which were recovered from annual blue grass and collected in Connecticut, share over 80% similarity with isolate 1 from bent grass collected in Connecticut (Fig. 1). This suggests the possibility that location is more important in determining the genetic relatedness among individual *C. graminicola* isolates than recovery from a similar host. Of 12 *C. graminicola* isolates from creeping bent grass, 7 in group III share 90% similarity and are more closely related to groups I and II (Fig. 1) than the other groups. Group IV (4 of 12 isolates) which also represents isolates from bent grass, shares over 70% similarity with group V, a group of isolates from annual blue grass, and about 64% similarity with group III, which represents isolates from the same host, bent grass. These observations suggest that a clear distinction between host-specific isolates, as detectable by RAPD markers, may not exist within *C. graminicola* from turf grass. However, it is clear that a close genetic relationship exists among the majority of isolates from the same host. Further intermating and cross-infection studies may provide more information about the genetic inter-relatedness of *C. graminicola* isolates from different turf grass hosts.

ACKNOWLEDGMENTS

We thank R. L. Nicholson, Purdue University, for supplying us with a stock culture of *C. graminicola*, isolated from maize, and *C. sublineolum*, isolated from sorghum.

LITERATURE CITED

1. Ali, M. E. K., and Warren, H. L. 1987. Physiological races of *Colletotrichum graminicola* on sorghum. Plant Dis. 71:402-404.
2. Bolton, A. T. and Cordukes, W. E. 1981. Resistance to *Colletotrichum graminicola* in strains of *Poa annua* and reaction of other turfgrasses. Can. J. Plant Pathol. 3:94-96.
3. Correll, J. C., and Guerber, J. C. 1993. Vegetative compatibility and virulence of the spinach anthracnose pathogen, *Colletotrichum dematium*. Plant Dis. 77:688-691.
4. Danneberger, T. K., Vargas, J. M., and Jones, A. L. 1984. A model for weather-based forecasting of anthracnose on annual blue grass. Phytopathology 74:448-451.
5. Forgey, W. M., Blanco, M. H., and Loegering, W. Q. 1978. Differences in pathological capabilities and host specificity of *Colletotrichum graminicola* on *Zea mays*. Plant Dis. Rep. 62:573-576.
6. Guthrie, P. A. I., Magill, C. W., Frederiksen, R. A., and Odvody, G. N. 1992. Random amplified polymorphic DNA markers: A system for identifying and differentiating isolates of *Colletotrichum graminicola*. Phytopathology 82:832-835.
7. Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. 1995. Ainsworth & Bisby's Dictionary of the Fungi, 8th ed. CAB International, Oxon, UK.
8. Luttrell, E. S. 1958. The perfect stage of *Helminthosporium turcicum*. Phytopathology 48:281-287.

Table 7. Frequency of potential diagnostic DNA bands present in *Colletotrichum graminicola* isolates from either creeping bent grass (CBG; total = 12) or annual blue grass (ABG; total = 9)^a

Primer	Band ^b	No. of isolates from CBG with band ^c	No. of isolates from ABG with band ^c
UBC 352	G	0/12	7/9 (17,18)
UBC 360	E	11/12 (4)	2/9 ****
UBC 360	F	0/12	5/9 (17,18,19,21)
UBC 360	K	8/12 (3,4,5,11)	0/9
UBC 397	B	6/12 (1,3,4,5,11,12)	0/9
UBC 397	G	8/12 (3,4,5,11)	0/9
UBC 339	B	7/12 (1,3,4,5,11)	0/9
UBC 339	M	6/12 (1,3,4,5,6,11)	0/9
UBC 391	D	7/12 (1,3,4,5,12)	0/9
UBC 391	J	1/12**	7/9 (17,18)
UBC 387	A	7/12 (1,3,4,5,11)	0/9
UBC 387	B	1/12***	5/9 (17,18)
UBC 387	K	7/12 (1,3,4,5,11)	0/9
UBC 387	M*	8/12 (3,4,5,11)	0/9
UBC 357	D	0/12	5/9 (17,18,19,21)
UBC 345	A	11/12 (1)	0/9
UBC 345	B	0/12	7/9 (19,21)
UBC 345	D	0/12	7/9 (19,21)
UBC 345	G	0/12	5/9 (17,18,19,21)
UBC 345	H	0/12	6/9 (17,19,21)

^a * = also present in corn; ** = present in isolate 11; *** = present in isolate 12; and **** = present in isolates 17 and 18.

^b Bands were assigned letter designations sequentially (beginning with A) from those closest to the origin of migration to those furthest away.

^c Exceptional isolate number lacking the diagnostic band.

9. Nicholson, R. L., and Warren, H. L. 1981. The issue of races of *Colletotrichum graminicola* pathogenic to corn. *Plant Dis.* 65:143-145.
10. Pande, S., Mughogho, L. K., Bandyopadhyay, R., and Karunakar, R. I. 1991. Variation in pathogenicity and cultural characteristics of sorghum isolates of *Colletotrichum graminicola* in India. *Plant Dis.* 75:778-783.
11. Politis, D. J. 1975. The identity and perfect state of *Colletotrichum graminicola*. *Mycologia* 67:56-62.
12. Rohlf, F. J. 1993. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, NY.
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1990. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
14. Sheriff, C., Whelan, M. J., Arnold, G. M., and Bailey, J. A. 1995. rDNA sequence analysis confirms the distinction between *Colletotrichum graminicola* and *C. sublineolum*. *Mycol. Res.* 99:475-478.
15. Shoemaker, R. A. 1955. Biology, cytology, and taxonomy of *Cochliobolus sativus*. *Can. J. Bot.* 33:562-575.
16. Smiley, R. W., Dernoeden, P. H., and Clark, B. B. 1992. *Compendium of Turfgrass Diseases*, 2nd ed. American Phytopathological Society, St. Paul, MN.
17. Smith, J. D., Jackson, N., and Woolhouse, A. R. 1989. *Fungal Diseases of Amenity Turfgrasses*, 3rd ed. E. and F. N. Spon, New York.
18. Sutton, B. C. 1968. The appressoria of *Colletotrichum graminicola* and *C. falcatum*. *Can. J. Bot.* 46:873-876.
19. Sutton, B. C. 1980. *The Coelomycetes: Fungi Imperfecti with Pycnidia, Acervuli, and Stromata*. Commonwealth Mycological Institute, Kew, Surrey, England.
20. Vaillancourt, L. J., and Hanau, R. M. 1992. Genetic and morphological comparisons of *Glomerella* (*Colletotrichum*) isolates from maize and from sorghum. *Exp. Mycol.* 16:219-229.
21. Wheeler, B. E. J. 1968. Fungal parasites of plants. Pages 179-210 in: *The Fungi III. The Fungal Population*. G. C. Ainsworth and A. S. Sussman, eds. Academic Press, New York.
22. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531-6535.